# Biological limits on nitrogen use for plant photosynthesis: a quantitative revision comparing cultivated and wild species

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## Summary

- The relationship between leaf photosynthesis and nitrogen is a critical production function for ecosystem functioning. Cultivated species have been studied in terms of this relationship, focusing on improving nitrogen (N) use, while wild species have been studied to evaluate leaf evolutionary patterns. A comprehensive comparison of cultivated vs wild species for this relevant function is currently lacking. We hypothesize that cultivated species show increased carbon assimilation per unit leaf N area compared with wild species as associated with artificial selection for resource-acquisition traits.
- We compiled published data on light-saturated photosynthesis ($A_{max}$) and leaf nitrogen ($LN_{area}$) for cultivated and wild species. The relationship between $A_{max}$ and $LN_{area}$ was evaluated using a frontier analysis (90$^{th}$ percentile) to benchmark the biological limit of nitrogen use for photosynthesis.
- Carbon assimilation in relation to leaf N was not consistently higher in cultivated species; out of 14 cultivated species, only wheat, rice, maize and sorghum showed higher ability to use N for photosynthesis compared with wild species.
- Results indicate that cultivated species have not surpassed the biological limit on nitrogen use observed for wild species. Future increases in photosynthesis based on natural variation need to be assisted by bioengineering of key enzymes to increase crop productivity.

## Introduction

Leaf photosynthesis is the main process of energy capture for the total biosphere (Lange *et al.*, 1987). Understanding ecosystem functioning requires analyzing photosynthesis performance of relevant plant groups from both natural ecosystems and agroecosystems. Light harvesting processes and electron transport, jointly with the enzymatic machinery of carbon (C) metabolism, require large investments in leaf nitrogen (N) in the form of protein (Hohmann-Marriott & Blankenship, 2011). Therefore, N is considered the main limiting nutrient for primary productivity for both agricultural and natural environments (LeBauer & Treseder, 2008). The relationship between leaf N and photosynthesis is a fundamental production-resource function for ecosystem functioning as photosynthesis also provides the energy for heterotrophic consumption (Field & Mooney, 1991; Vitousek *et al.*, 1997). Here we describe the relationship between leaf, light-saturated, photosynthesis ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$) and N per unit leaf area ($LN_{area}$; g $m^{-2}$) for cultivated and wild species using a database compiled from previously published data.

Modern agriculture depends on the productivity of a limited set of species (Geps, 2002; Ross-Ibarra *et al.*, 2007). Cultivated and wild species have been independently characterized for their ability to use leaf N for photosynthesis. Research on cultivated species focused on practical aspects related to improved N-use efficiency for higher yield, food security, and reduced pollution (Muchow & Sinclair, 1994; Peng *et al.*, 1995; Dreccer *et al.*, 2000; Cassman *et al.*, 2003). Studies evaluating wild species, on the other hand, have usually tested whether the relationship between $A_{max}$ and $LN_{area}$ reflect evolutionary patterns associated with resource availability and environmental constraints (Field & Mooney, 1991; Poorter & Evans, 1998; Wright *et al.*, 2004, 2005; Hassiotou *et al.*, 2010). Comparing this production function provides a unique opportunity to test whether there has been improvement in the ability to utilize N in cultivated species beyond what is observed for wild species. A comprehensive comparison including major cultivated species upon which human food supply relies is currently lacking.

The $A_{max}$–$LN_{area}$ relationship is modified by different environmental factors and/or other nutrient limitations. Reduced water availability impacts this relationship mainly as a result of stomatal limitations for C fixation (e.g. Flexas & Medrano, 2002). Reich *et al.* (2009) also demonstrated that the initial slope of the relationship decreases with phosphorus deficiency associated with limitations in ribulose-1,5-bisphosphate regeneration. Peterson *et al.* (1999) showed that increased atmospheric $CO_2$ concentration increased the response of $A_{max}$ to $LN_{area}$. Atkinson *et al.* (2010) determined that growing temperature altered the scaling

of the relationship. According to these examples, significant scattering is expected when plotting $A_{max}$ vs $LN_{area}$ data compiled from independent studies. Under this scenario, a quantile regression approach would be useful to isolate the impact of $LN_{area}$ on $A_{max}$ in situations where other factors are not limiting. This analysis, performed at the 90$^{th}$ percentile for $A_{max}$, will allow the biological limit of the $A_{max}$–$LN_{area}$ relationship to be determined for different cultivated and wild species. The relationship cannot change above that limit, but may be reduced when other factors are limiting. Therefore, using a quantile regression approach we will set the limits of this production-resource function (Cade & Noon, 2003; Archontoulis & Miguez, 2015). A quantile regression approach has been developed and successfully utilized to establish the maximum return of water invested in transpiration for crops (i.e. French & Schultz, 1984; Sadras & Angus, 2006). This study is the first to benchmark the upper limit for N use at the leaf level for different cultivated and wild species.

The ratio between $A_{max}$ and $LN_{area}$ defines the photosynthetic N-use efficiency as the amount of C fixed per unit of N invested in a leaf (PNUE; Poorter & Evans, 1998). As demonstrated by Drecer *et al.* (2000), PNUE varies as a function of $LN_{area}$. Obtaining the PNUE from the $A_{max}$–$LN_{area}$ relationship will also serve to benchmark the maximum PNUE for cultivated and wild species.

The objectives of this paper were: to determine the biological limit of the relationship between $A_{max}$ and $LN_{area}$ by benchmarking the parameters of the function defined in Sinclair & Horie (1989) for major cultivated and wild species; to contrast the relationship between PNUE and $LN_{area}$ for major cultivated and wild species; and to determine the maximum PNUE and the $LN_{area}$ at which it occurs for cultivated and wild species. We hypothesized that cultivated species will have increased photosynthetic N-use efficiency compared with wild species as possibly associated with artificial selection for resource-acquisition traits (Denison, 2009); this difference would be higher for annual cultivated species compared with cultivated perennials as the latter have a longer intergenerational period and therefore reduced cycles of selection.

## Materials and Methods

### Cultivated database compiled from published literature

To obtain data on cultivated species, a database was built from *ad hoc* species-specific bibliographic search. The search was oriented to papers on ecophysiological plant responses of different cultivated species from outdoor or glasshouse experiments at ambient $CO_2$ pressure. The papers reported the C exchange rate at light-saturated conditions ($A_{max}$) determined by infrared gas analyzer equipment (e.g. Li-Cor 6400 instrument, Lincoln, NE, or similar), and leaf N content on a leaf area basis ($LN_{area}$) in such a way as to reproduce these variables as μmol $CO_2$ $m^{-2}$ $s^{-1}$ and g N $m^{-2}$, respectively. Light-saturated conditions were reported in all cases, with light intensity ranging from 1200 to 2000 μmol $m^{-2}$ $s^{-1}$ depending on the species. We excluded measures in hydroponic conditions or with increases in ambient $CO_2$ pressure. We obtained 48 papers that studied this relationship for 14 cultivated species, and we generally obtained three to 10 papers for each species (Supporting Information Table S1). The cultivated species were three $C_3$ winter cereal crops (*Triticum aestivum* (wheat), *Oryza sativa* (rice), and *Hordeum vulgare* (barley)), two summer $C_4$ cereal crops (*Zea mays* (maize) and *Sorghum bicolor* (sorghum)), five $C_3$ dicotyledonous annual crops (*Glycine max* (soybean), *Helianthus annuus* (sunflower), *Gossypium hirsutum* (cotton), *Brassica napus* (rapeseed), and *Solanum tuberosum* (potato)) and four cultivated trees (*Malus domestica* (apple), *Prunus dulcis* (almond), *Prunus persica* (peach), and *Citrus* × *paradise* (grapefruit)). For each paper we extracted the results from published scatter plots or data tables as paired observations of $A_{max}$ and $LN_{area}$, totaling between 25 and 513 paired observations according to the species. A total of 2874 paired observations of $A_{max}$ and $LN_{area}$ for cultivated species were compiled from a period spanning from 1980 to 2012 (Tables S1, S2).

### Wild database compiled from published literature

The search was oriented to papers on ecophysiological plant responses of different wild species with similar keywords as for the construction of the 'cultivated' database. Included papers reported outdoor or glasshouse studies that measured $A_{max}$ and $LN_{area}$ also in such a way to reproduce these variables as μmol $CO_2$ $m^{-2}$ $s^{-1}$ and g N $m^{-2}$, respectively. All papers included radiation measures and/or stated that $A_{max}$ rate was measured under light-saturated conditions, which in most of cases ranged between 800 and 2000 μmol $m^{-2}$ $s^{-1}$. We excluded measures in hydroponic conditions or with modifications in ambient $CO_2$ pressure. The life form (e.g. trees, grasses, dicotyledonous herbs, etc.) and C metabolism (i.e. $C_3$ or $C_4$) of the wild species were recorded to facilitate comparison against cultivated species characteristics. We obtained 35 papers that studied this relationship for wild species with measurements from the period 1981–2011 and across five continents (Tables S1, S2). In total we obtained 2326 paired observations of $A_{max}$ and $LN_{area}$ for wild species. The wild database included data from the GLOPNET database (Wright *et al.*, 2004).

For both cultivated and wild datasets, observations of $LN_{area}$ > 4 g N $m^{-2}$ associated with low C exchange rates were eliminated as possible outliers or species having specific adaptations for high investment in N with low return in terms of $A_{max}$ (e.g. species producing alkaloids). These eliminated data represented < 0.1% of total revised data. All data used in the analysis were compiled into a unified database (Table S2).

### Modeling the $A_{max}$ vs $LN_{area}$ relationship and statistical analysis

The relationship between $A_{max}$ and $LN_{area}$ was modeled following the logistic model proposed by Sinclair & Horie (1989):

$$A_{max} = \alpha \cdot \left[\frac{2}{\left(1 + e^{(-\beta(LN_{area} - \gamma))}\right)}\right] - 1 \qquad \text{Eqn 1}$$

where $\alpha$ is the asymptotic $A_{max}$ at high $LN_{area}$ values, $\beta$ represents the curvature of the response between $A_{max}$ and $LN_{area}$, and $\gamma$

indicates the $LN_{area}$ value at which $A_{max}$ is zero. This equation has the versatility to accommodate linear trends if needed through reduced $\beta$ values. Data for the cultivated and wild species were analyzed by quantile regression for estimating parameters of the Sinclair & Horie (1989) model; conditional quantile functions at the 90th percentile from the QUANTREG package (Koenker, 2015) were used (R Core Team, 2013). After fitting models, goodness-of-fit tests such as the likelihood ratio test and a pseudo-$R^2$ were conducted ($R_1$; Koenker & Machado, 1999). The $R_1$ metric is a local measure of goodness of fit at a particular quantile. It compares the sum of weighted deviations from the model of interest with the same sum from a model in which only the intercept appears (null model). In addition, approximate confidence intervals (95%) for each parameter (i.e. $\alpha$, $\beta$ and $\gamma$) were built by bootstrapping (Koenker & Park, 1996; Koenker, 2005). The comparison among crops and wild species (see next section) was based on the punctual and interval estimates for each model parameter.

A leaf area-based analysis of the relationship between leaf photosynthesis and N was chosen as the alternative leaf mass-based approximation (i.e. $g^{-1}$ leaf). There were two reasons for this decision. First, as solar radiation capture and C assimilation are intrinsically area-based processes, working on leaf area provides a suitable resource-harvesting framework to better understand N impacts on C capture (Field & Mooney, 1991), which is the major objective of our work. The mass-based analysis has been more appropriate to understand the economics of C and N allocation in species from contrasting habitats (Wright *et al.*, 2004). Second, the amount of area-based data is substantially higher than the amount of mass-based data. Therefore, focusing on mass-based data would have reduced the comprehensiveness of the database by reducing the total number of cases available for analysis.

Photosynthetic N-use efficiency was calculated as the quotient ratio between $A_{max}$ and $LN_{area}$ using the predicted values of the benchmarked model. PNUE was calculated only for the observed range of $LN_{area}$ values. Maximum PNUE and $LN_{area}$ at maximum PNUE were determined by first-degree derivation of the predicted relationship between PNUE and $LN_{area}$. Confidence intervals for the maximum PNUE across cultivated and wild species were built by propagating the uncertainty of estimates from the original quantile regression on $A_{max}$ and $LN_{area}$, and assuming a lack of error in the punctual estimation of the $LN_{area}$ for maximum PNUE. To conduct this analysis we used the package PROPAGATE (Spiess, 2014) from the R environment, and the comparison among cultivated and wild species was based on the punctual and interval estimates for the maximum PNUE.

### Comparison of cultivated and wild species

Individual cultivated species were compared against the best equivalent wild species functional groups. For instance, cultivated $C_3$ winter cereals (i.e. wheat, rice, and barley) were compared against wild $C_3$ graminoids. The $C_4$ summer cereal crops (i.e. maize and sorghum) were compared against $C_4$ wild graminoids. The $C_3$ dicotyledonous annual crops (i.e. soybean, sunflower, rapeseed, cotton, and potato) were compared against the wild $C_3$ dicotyledonous herbaceous plants. The perennial fruit/nut cultivated trees were compared against $C_3$ woody plants. The rationale behind not grouping the cultivated species is that it is highly relevant analyzing these 14 species individually, as they represent most of the primary production worldwide. By grouping the wild species, we sought to determine the limits of PNUE for wild species as a reference group, regardless of the individual species. For each cultivated species and wild functional group, we built the respective scatter plot relating $A_{max}$ and $LN_{area}$.

## Results

### Cultivated $C_3$ winter cereals vs wild $C_3$ graminoids

Sinclair & Horie (1989)'s model fitted for the $C_3$ cultivated cereals and $C_3$ wild graminoids was significant, with $R_1$ ranging from 0.28 to 0.64 (Table 1). Wheat and rice had a lower $A_{max}$ at maximum $LN_{area}$ ($\alpha$ in Eqn 1) compared to the wild $C_3$ graminoids, while barley was not different from wild species (Table 2; Fig. 1a–c). The curvature of the relation between $A_{max}$ and $LN_{area}$ ($\beta$ in Eqn 1) was higher for wheat and rice than for the wild $C_3$ graminoids; there was no difference in this parameter between barley and the wild counterparts (Table 2). The minimum $LN_{area}$ for $A_{max} > 0$ ($\gamma$ parameter, Eqn 1) was significantly higher for barley when compared to $C_3$ wild graminoids (Table 2).

Wheat and rice had higher maximum PNUE than the wild species, attained at relatively low $LN_{area}$ (~1 g N $m^{-2}$) (Table 2;

**Table 1** Summary of goodness-of-fit tests for different wild plant functional types and related cultivated species based on the log-likelihood ratio and respective chi-squared tests, and pseudo-$R^2$ measures ($R_1$)* for the Sinclair & Horie (1989) model at the 90th percentile

| Wild groups and related crops | Log-likelihood null model | Log-likelihood Sinclair | $\chi^2_{(2)}$ | *P*-value | $R_1$ |
|---|---|---|---|---|---|
| $C_3$ graminoids | −1390.5 | −1207.08 | 365.4 | <0.0001 | 0.43 |
| Wheat | −829.7 | −682.7 | 294.1 | <0.0001 | 0.50 |
| Rice | −1084.3 | −976.5 | 215.5 | <0.0001 | 0.28 |
| Barley | −618.6 | −456.3 | 324.6 | <0.0001 | 0.64 |
| $C_4$ graminoids | −271.65 | −269.48 | 4.33 | 0.1146 | 0.03 |
| Maize | −1136.7 | −1079.5 | 114.4 | <0.0001 | 0.20 |
| Sorghum | −539.4 | −462.8 | 153.1 | <0.0001 | 0.43 |
| $C_3$ dicot herbs | −2347.3 | −2054.3 | 586.1 | <0.0001 | 0.41 |
| Soybean | −1420.2 | −1259.5 | 321.2 | <0.0001 | 0.36 |
| Sunflower | −1292.3 | −1154.2 | 276.1 | <0.0001 | 0.33 |
| Cotton | −1949.5 | −1913.1 | 72.7 | <0.0001 | 0.07 |
| Rapeseed | −563.5 | −528.8 | 69.5 | <0.0001 | 0.22 |
| Potato | −275.3 | −216.9 | 116.8 | <0.0001 | 0.49 |
| $C_3$ trees | −4061.2 | −3648 | 826.4 | <0.0001 | 0.31 |
| Apple | −73.6 | −39.8 | 67.6 | <0.0001 | 0.74 |
| Almond | −272.3 | −245.5 | 56.3 | <0.0001 | 0.28 |
| Peaches | −254.9 | −218.9 | 71.8 | <0.0001 | 0.36 |
| Grapefruit | −181.1 | −163.9 | 34.1 | <0.0001 | 0.22 |

*$R_1$ is described as a local measure of goodness of fit at the particular quantile by comparing the sum of weighted deviations for the model of interest with the same sum from a model in which only the intercept appears.

**Table 2** Parameters relating light-saturated photosynthesis rate ($A_{max}$) and leaf nitrogen content ($LN_{area}$) for $C_3$ monocots

| Functional group and related crops | Fitted parameters (95% CI) | | | Max. PNUE (95% CI) | $LN_{area}$ at max PNUE |
|---|---|---|---|---|---|
| | $\alpha$ | $\beta$ | $\gamma$ | | |
| $C_3$ wild graminoids | 61.3 (43.7–78.8) | 0.68 (0.41–0.95) | 0.15 (0.07–0.22) | 17.6 (14.2–18.3) | 1.38 |
| Wheat | 36.3 (32.8–39.8) | 1.61 (1.19–2.02) | 0.23 (0.11–0.34) | 20.1 (18.1–21.2) | 1.01 |
| Rice | 33.2 (28.3–38.1) | 2.01 (1.06–2.94) | 0.22 (0.02–0.42) | 21.74 (17.6–22.6) | 0.90 |
| Barley | 59.1 (44.8–73.4) | 0.96 (0.59–1.32) | 0.31 (0.25–0.38) | 20.2 (16.9–20.7) | 1.56 |

Summary of quantile regression analysis (90$^{th}$ percentile) between $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) and $LN_{area}$ (g N $m^{-2}$) for $C_3$ wild graminoids and related crops (wheat, rice and barley). Numbers indicate the punctual estimates for the parameters of the Sinclair & Horie (1989) model model ($\alpha$, maximum $A_{max}$; $\beta$, the curvature of the relationship; and $\gamma$, minimum N leaf area for photosynthesis), as well the maximum photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$), and the punctual $LN_{area}$ at maximum PNUE. Values between parentheses are the confidence intervals of the estimates.

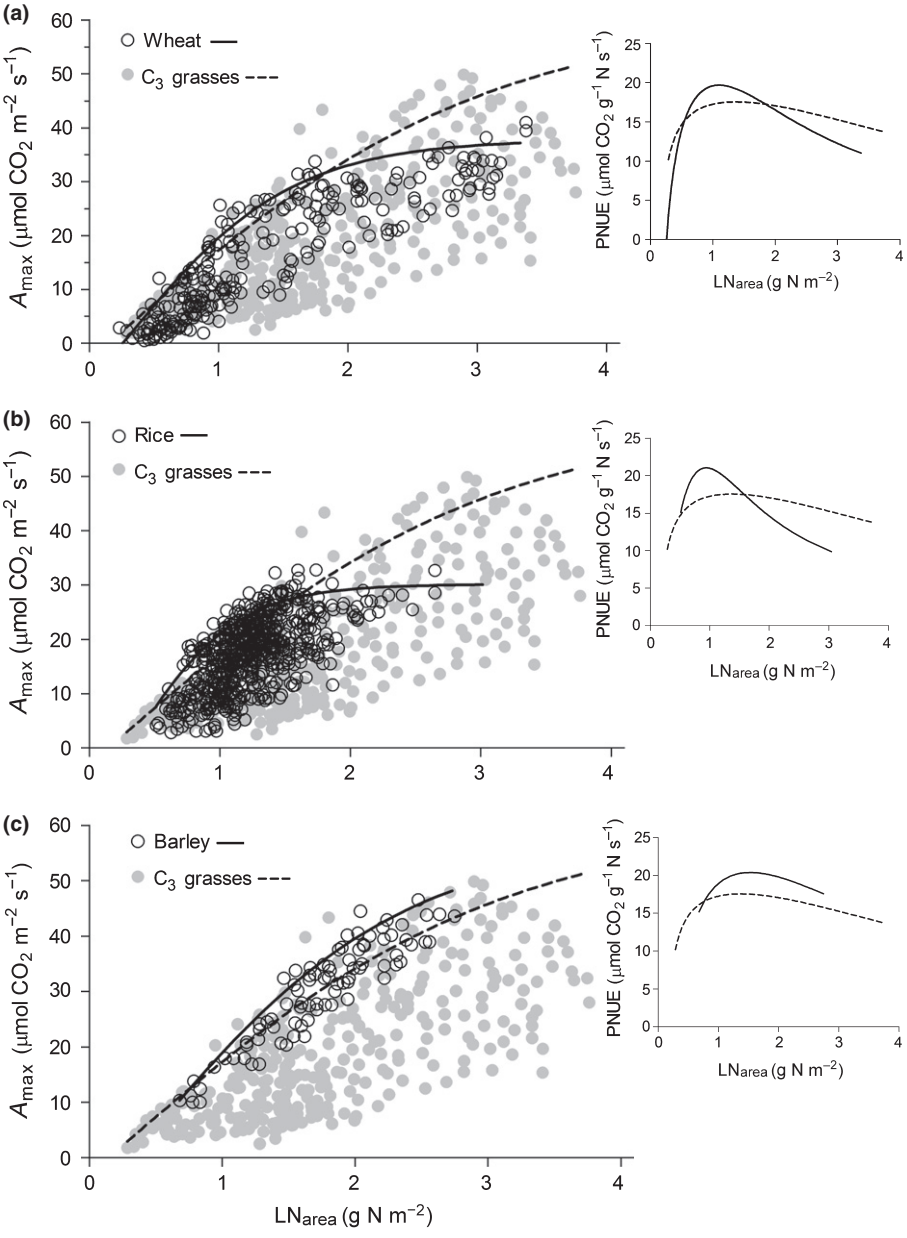


**Fig. 1** Quantile regression models for the relationship between the light-saturated photosynthesis rate ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$) and the leaf nitrogen content ($LN_{area}$; g N $m^{-2}$) for wild $C_3$ graminoids (dashed line and gray markers) and related crop species (solid line and white markers): (a) wheat; (b) rice; (c) barley. Insets: the predicted photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$) along the same leaf nitrogen content gradient according to the fitted model.

Fig. 1a,b insets); the barley maximum PNUE was attained at a higher $LN_{area}$ (1.56 g N $m^{-2}$) compared with the other cultivated species, and was not significantly different from the wild species. Wheat and rice were more efficient than wild $C_3$ graminoids at $LN_{area} < 1.5$ g N $m^{-2}$ (Table 2; Fig. 1 insets).

### Cultivated $C_4$ cereals vs $C_4$ wild graminoids

Sinclair & Horie's model fitted for $C_4$ cultivated species was significant, with $R_1 = 0.2$ for maize and $R_1 = 0.43$ for sorghum; however, the model fitted for the wild $C_4$ graminoid species was not significantly different from the null model represented only by the intercept ($\alpha = 42.3$) (Table 1). Maize $\alpha$ was significantly higher compared to the $C_4$ wild graminoids (Table 3). Sorghum had a significantly higher $\alpha$ than maize and the wild $C_4$ counterparts (Table 3; Fig. 2b). Owing to the high uncertainty in the estimation of $\beta$ for the $C_4$ wild species, there were no significant differences for this parameter between cultivated and wild $C_4$ grasses. The $\gamma$ parameter for maize and sorghum was not different from zero, and was not different from wild $C_4$ estimates (Table 3).

Maize and sorghum maximum PNUE values were approximately half that of wild $C_4$ species (Table 3). These maximums were also achieved near to the origin (0.28–0.34 g N $m^{-2}$, Table 3; Fig. 2 insets). As wild and cultivated $C_4$ species present $\gamma$ estimates $< 0$, the relationship between PNUE and $LN_{area}$ showed a decreasing trend from their maximum (Fig. 2 insets). Wild $C_4$ PNUE was higher than that for the $C_4$ crops below *c.* 1 g N $m^{-2}$ leaf; above this value, differences between crops and wild $C_4$ graminoids were not evident (Fig. 2 insets).

### Cultivated $C_3$ dicotyledonous annuals vs wild $C_3$ dicotyledonous herbaceous

Sinclair & Horie's models fitted for cultivated $C_3$ dicotyledonous species and $C_3$ wild herbs were significant, with $R_1$ in the range 0.07–0.49 (Table 1). The $\alpha$ parameter for soybean, sunflower, and rapeseed was not different from that for wild $C_3$ herbs (Table 4; Fig. 3a,b,d); cotton and potato, on the other hand, had a lower $\alpha$ than wild $C_3$ herbs (Table 4; Fig. 3c,e). A higher $\beta$ was observed for sunflower, cotton and potato than for the wild $C_3$ herbs (Table 4); soybean and rapeseed were not significantly different from their wild counterpart. For all the $C_3$ dicotyledonous crops, except potato, the minimum $LN_{area}$ for photosynthesis ($\gamma$) was higher than zero (0.238–0.615 g N $m^{-2}$) and significantly higher than that for wild $C_3$ herbs (Table 4; Fig. 3). However, the $\gamma$ estimate for potato was not different from zero and not different from that for wild $C_3$ herbs.

The maximum PNUE values for sunflower, cotton, and rapeseed were not different from those for the wild species (Table 4; Fig. 3b–d insets). Soybean and potato have the lowest maximum PNUE, with a significant reduction of 39–47% compared with wild species (Table 4; Fig. 3a,e insets). The difference in the PNUE–$LN_{area}$ relationship between cultivated and wild species was a result of the remarkable difference in the $\gamma$ parameter. The maximum PNUE occurred at a relatively low $LN_{area}$ (0.23 g N $m^{-2}$) in wild species, while the maximum PNUE for the cultivated species was observed at intermediate values (0.65–1.72 g N $m^{-2}$) (Table 4; Fig. 3 insets).

### Cultivated fruit/nut trees vs $C_3$ woody plants

All Sinclair & Horie's models fitted for cultivated $C_3$ trees and wild woody $C_3$ species were significant, with $R_1$ ranging from 0.22 to 0.74 (Table 1). Grapefruit was the cultivated $C_3$ tree with the lowest $\alpha$ compared with both the wild trees and the remaining of the cultivated ones (Table 5; Fig. 4d). Apple, almond, and peach were not different from the $C_3$ wild trees for the $\alpha$ parameter (Table 5; Fig. 4a–c). For all $C_3$ crop trees except almond, the $\beta$ parameter was not different compared with the wild trees; almond, on the other hand, had a significantly higher $\beta$ than did the wild trees (Table 5). For all the cultivated $C_3$ trees, $\gamma$ was significantly higher compared with the $C_3$ wild trees (Table 5).

Owing to the negative $\gamma$ estimate of $C_3$ wild trees, the PNUE for this group was highest at infinitesimally low $LN_{area}$ (Fig. 4). Wild trees had a maximum PNUE of 31.6 μmol $CO_2$ $g^{-1}$ N $s^{-1}$, attained at 0.11 g N $m^{-2}$ (Table 5). The maximum PNUE of cultivated $C_3$ trees was, on average, 8.6 μmol $CO_2$ $g^{-1}$ N $s^{-1}$, attained at an average $LN_{area}$ of 1.7 g N $m^{-2}$. For all cultivated trees, the values of maximum PNUE and $LN_{area}$ at highest

**Table 3** Parameters relating light-saturated photosynthesis rate ( $A_{max}$) and leaf nitrogen content ($LN_{area}$) for $C_4$ monocots

| Functional group and related crops | Fitted parameters (CI 95%) | | | Max. PNUE (CI 95%) | $LN_{area}$ at max. PNUE |
|---|---|---|---|---|---|
| | $\alpha$ | $\beta$ | $\gamma$ | | |
| $C_4$ wild graminoids | 42.3 (38.9–45.8) | 6.25 (−23.1–35.6) | −0.05 (−4.7–4.9) | 206.2 (188.8–222.3) | 0.21 |
| Maize | 52.1 (45.4–58.9) | 2.40 (0.81–4) | −0.09 (−0.43–0.25) | 74.7 (68.2–81.9) | 0.28 |
| Sorghum | 59.4 (53.4–64.9) | 1.69 (0.79–2.58) | −0.17 (−0.6–0.26) | 71.0 (49.8–86) | 0.34 |

Summary of quantile regression analysis (90th percentile) between $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) and $LN_{area}$ (g N $m^{-2}$) for $C_4$ wild graminoids and related crops (maize and sorghum). Numbers indicate the punctual estimates for the parameters of the Sinclair & Horie (1989) model ($\alpha$, maximum $A_{max}$; $\beta$, the curvature of the relationship; and $\gamma$, minimum $LN_{area}$ for photosynthesis), as well the maximum photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$), and the punctual $LN_{area}$ at maximum PNUE. Values between parentheses are the confidence intervals of the estimates.

PNUE were lower and higher, respectively, than those for the wild trees. However, both groups explored a different range of $LN_{area}$ (Fig. 4). The comparison of the relationship between PNUE and $LN_{area}$ in the *x*-axis range shared by the cultivated and wild trees showed a near-identical response for these groups in terms of PNUE performance across varying $LN_{area}$ (Fig. 4).

## Discussion

The development of modern agriculture moved plants from wild environments to cultivated lands with higher resource abundance and lower pest/disease pressure (Evans, 1993). Plant strategy theory predicts trajectories during crop evolution shifting from a

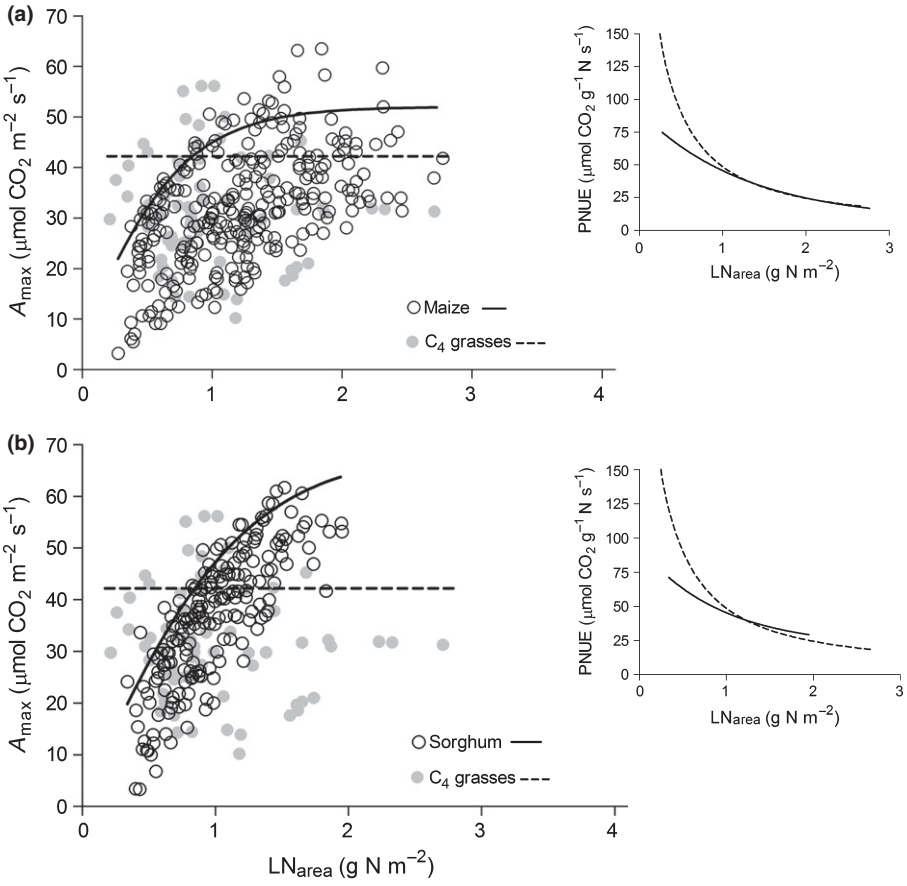


**Fig. 2** Quantile regression models for the relationship between the light-saturated photosynthesis rate ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$) and the leaf nitrogen content ($LN_{area}$; g N $m^{-2}$) for wild $C_4$ graminoids (dashed line and gray markers) and related crop species (solid line and white markers): (a) maize; (b) sorghum. Insets: the predicted photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$) along the same leaf nitrogen content gradient according to the fitted model.

**Table 4** Parameters relating light-saturated photosynthesis rate ( $A_{max}$) and leaf nitrogen content ($LN_{area}$) for $C_3$ dicot herbs

| Functional group and related crops | Fitted parameters (95% CI) | | | Max. PNUE (95% CI) | $LN_{area}$ at max. PNUE |
|---|---|---|---|---|---|
| | $\alpha$ | $\beta$ | $\gamma$ | | |
| $C_3$ wild herbs | 51.3 (45.8–56.8) | 0.87 (0.65–1.08) | −0.02 (−0.11–0.08) | 23.9 (15.4–28.1) | 0.23 |
| Soybean | 55.3 (41.8–68.7) | 0.65 (0.39–0.91) | 0.24 (0.13–0.35) | 14.4 (10.8–14.9) | 1.72 |
| Sunflower | 46.0 (42.4–49.6) | 1.33 (1.06–1.6) | 0.28 (0.12–0.43) | 21.3 (19.2–22.2) | 1.15 |
| Cotton | 40.0 (37.9–42.2) | 2.27 (1.23–3.3) | 0.62 (0.31–0.93) | 20.4 (15.4–22.5) | 1.46 |
| Rapeseed | 46.9 (35.6–58.1) | 1.92 (0.73–3.12) | 0.38 (0.23–0.53) | 25.7 (12.7–28.9) | 1.19 |
| Potato | 20.7 (17.9–23.4) | 1.44 (0.95–1.93) | 0.07 (−0.1–0.24) | 12.6 (10.3–13.9) | 0.65 |

Summary of quantile regression analysis (90$^{th}$ percentile) between $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) and $LN_{area}$ (g N $m^{-2}$) for $C_3$ wild dicotyledonous herbs and related crops (soybean, sunflower, cotton, rapeseed, and potato). Numbers indicate the punctual estimates for the parameters of the Sinclair & Horie (1989) model ($\alpha$, maximum $A_{max}$; $\beta$, the curvature of the relationship; and $\gamma$, minimum $LN_{area}$ for photosynthesis), as well the maximum photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$), and the punctual $LN_{area}$ at maximum PNUE. Values between parentheses are the confidence intervals of the estimates.

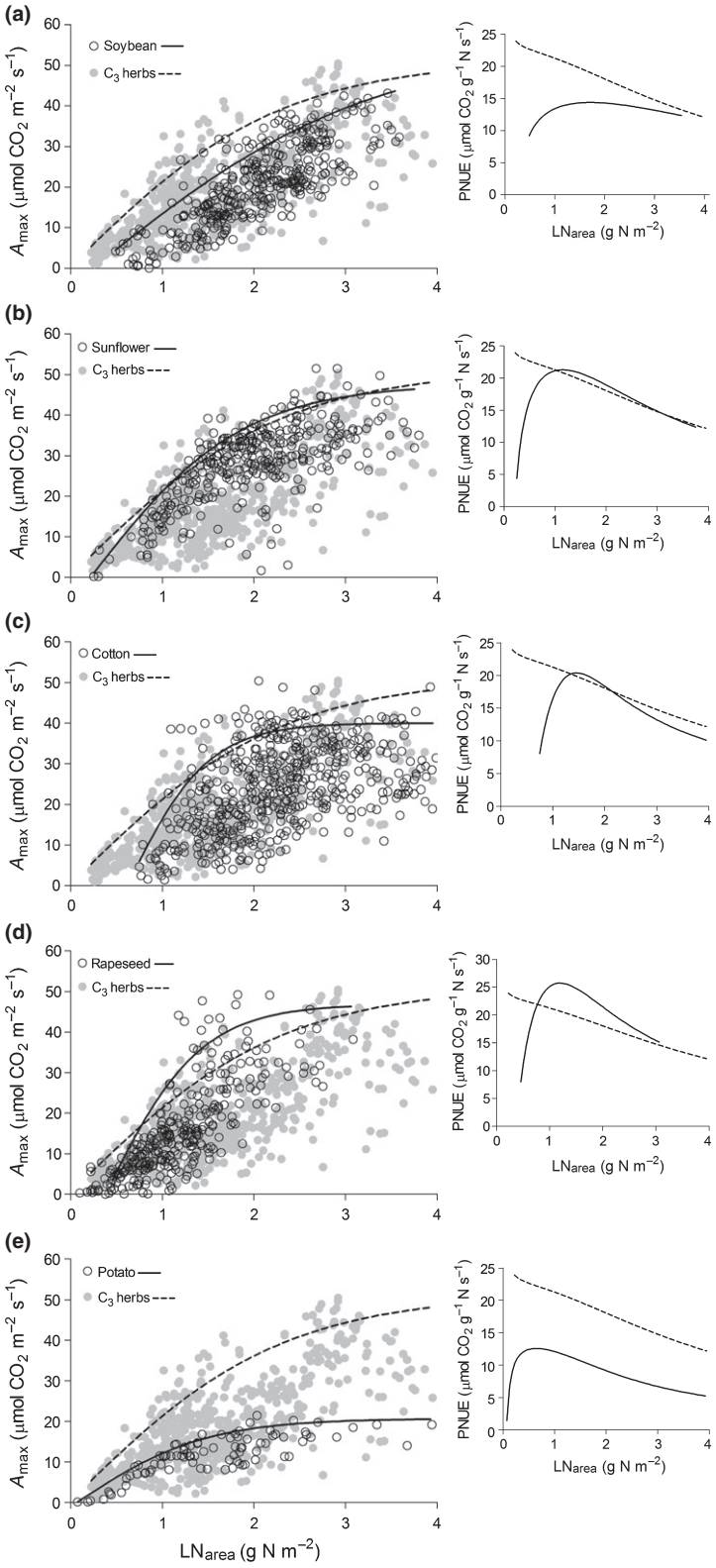


**Fig. 3** Quantile regression models for the relationship between the light-saturated photosynthesis rate ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$) and the leaf nitrogen content ($LN_{area}$; g N $m^{-2}$) for wild $C_3$ dicotyledonous herbs (dashed line and gray markers) and related crop species (solid line and white markers): (a) soybean; (b) sunflower; (c) cotton; (d) rapeseed; (e) potato. Insets: the predicted photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$) along the same leaf nitrogen content gradient according to the fitted model.

resource-conservation to a resource-acquisition suite of traits (Denison, 2009). In this sense, we expected to see a high proportion of cultivated species surpassing the wild species for the $A_{max}$–$LN_{area}$ relationship. However, our findings support the concept that the cultivated species have not surpassed the biological limit of N use for plant photosynthesis observed for wild species. It can be speculated that potential differences between cultivated and wild species are attributed to crop domestication, artificial selection, and/or cultivation environment. Testing some of these hypotheses requires a more targeted comparison between

**Table 5** Parameters relating light-saturated photosynthesis rate ( $A_{max}$) and leaf nitrogen content ($LN_{area}$)for $C_3$ dicot trees

| Functional group and related crops | Fitted parameters (CI 95%) | | | Max. PNUE (95% CI) | $LN_{area}$ at max. PNUE |
|---|---|---|---|---|---|
| | $\alpha$ | $\beta$ | $\gamma$ | | |
| $C_3$ wild trees | 30.5 (19.2–41.8) | 0.54 (0.26–0.82) | −0.27 (−0.42 to −0.13) | 31.6 (17.8–36.1) | 0.11 |
| Apple | 23.2 (19.3–27.1) | 0.84 (0.45–1.23) | 0.23 (−0.10–0.57) | 7.5 (6.3–7.9) | 1.46 |
| Almond | 22.9 (19.9–25.8) | 4.13 (1.55–6.71) | 1.63 (1.53–1.74) | 8.8 (5.5–9.2) | 2.35 |
| Peaches | 23.7 (18.1–29.3) | 1.22 (0.46–1.97) | 0.39 (0.08–0.71) | 9.3 (6.5–9.7) | 1.52 |
| Grapefruit | 16.6 (14.8–18.3) | 2.02 (0.16–3.88) | 0.51 (−0.08–1.11) | 8.6 (2.1–9.4) | 1.46 |

Summary of quantile regression analysis (90$^{th}$ percentile) between $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) and $LN_{area}$ (g N $m^{-2}$) for $C_3$ wild trees and related cultivated species (apple, almond, peaches, and grapefruit). Numbers indicate the punctual estimates for the parameters of the Sinclair & Horie (1989) model ($\alpha$, maximum $A_{max}$; $\beta$, the curvature of the relationship; and $\gamma$, minimum $LN_{area}$ for photosynthesis), as well the maximum photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$), and the punctual $LN_{area}$ at maximum PNUE. Values between parentheses are the confidence interval of the estimates.

cultivated species and their specific ancestors. For example, in a comprehensive screening of cultivated vs wild ancestors, Milla *et al.* (2015) showed that wild progenitors of modern crops were already high resource-acquisition and utilization strategists. Preece *et al.* (2015) also showed that wild progenitors did not have any advantage in terms of resource acquisition compared with wild plants that were not selected for domestication. Similarly, higher PNUE has been observed in undomesticated *Glycine soja* compared with the domesticated *Glycine max* cultivars (Rotundo & Borrás, 2016). Domestication seems to have involved changes in other specific traits associated with seed shedding, biomass partitioning, and sensitivity to photoperiod (Ulukan, 2009).

Our evaluation provides parameters for the potential relationship between $A_{max}$ and $LN_{area}$ for major cultivated species and wild plant functional types, representing two major improvements on previous efforts to compile leaf photosynthesis data. First, previous comparisons focused on smaller sets of cultivated species. Evans (1989) compared only wheat and rice against a set of wild species. Wullschleger (1993) characterized di- and monocots crops as individual groups. Kattge *et al.* (2009) compared wild functional groups and $C_3$ cultivated species as a group. Our study is unique in that it reports a comparison of 14 major cultivated species against equivalent wild functional groups. Second, compiling a diverse set of data and performing a unique analysis are always challenging as data were, most probably, obtained under different environmental conditions. The frontier analysis followed here, set at the 90$^{th}$ percentile of the $A_{max}$–$LN_{area}$ relationship, provides the opportunity to eliminate the influence of any additional factor that is reducing the ability of a leaf to fix C (e.g. climatic conditions, nutrients other than N, etc.). The quantile regression approach, entirely novel to this area, allowed us to benchmark the potential ability to use N for C fixation in major cultivated species for world food and feeding and wild plants from natural ecosystems.

The difference between cultivated and wild species for the parameters of the $A_{max}$–$LN_{area}$ relationship depended on each particular parameter. First, in terms of $A_{max}$ at saturating $LN_{area}$ ($\alpha$), only maize and sorghum surpassed the wild species; it was lower in wheat, rice, cotton, potato, and grapefruit, and not different in the remaining eight cultivated species. Operating at high $LN_{area}$ (and high $A_{max}$) would not be evolutionary stable as this is frequently associated with reduced leaf area, reduced light interception, and reduced fitness (yield) (Sinclair & Horie, 1989). Second, the curvature of the relationship ($\beta$) was higher in five cultivated species (wheat, rice, sunflower, cotton, potato, and almond), compared with the wild counterparts. An increased curvature is generally associated with increased $A_{max}$ at intermediate $LN_{area}$ values. Selecting for cultivated populations having increased $A_{max}$ at intermediate values of $LN_{area}$ would not have costs in terms of reduced LAI that may limit light interception and it could be a trait selectable for increased yields. Finally, the minimum amount of $LN_{area}$ for photosynthesis was higher compared with wild species in all but five cultivated species that did not differ from their wild counterparts (rice, wheat, maize, sorghum, and potato). The higher $\gamma$ commonly found in these cultivated species may be associated with relaxed herbivory pressure which allows for more N-expensive leaves that are protected via chemical applications (Coley *et al.*, 1985). An alternative explanation is that the wild database also incorporated species from N-limited environments with inherent low $LN_{area}$ (Chapin, 1980).

With few exceptions, cultivated species PNUE values were inferior to, or not different from, those of the wild species. Wheat and rice had a steeper initial slope than did the wild species, determining a high PNUE at relatively low $LN_{area}$ (< 2 g N $m^{-2}$). In general, $LN_{area}$ changes from early stages to maturity range from 2.0 to 0.9 g N $m^{-2}$ and from 1.7 to 0.8 g N $m^{-2}$ in wheat and rice, respectively (Ohsumi *et al.*, 2007; Bertheloot *et al.*, 2008). These ranges match well those $LN_{area}$ values where PNUE tends to be highest for those crops. The high $A_{max}$ observed in both crops may be caused by greater N allocation to Rubisco (Makino *et al.*, 1992). Whether the higher PNUE observed in wheat and rice than in the wild $C_3$ grasses is also going to be valid

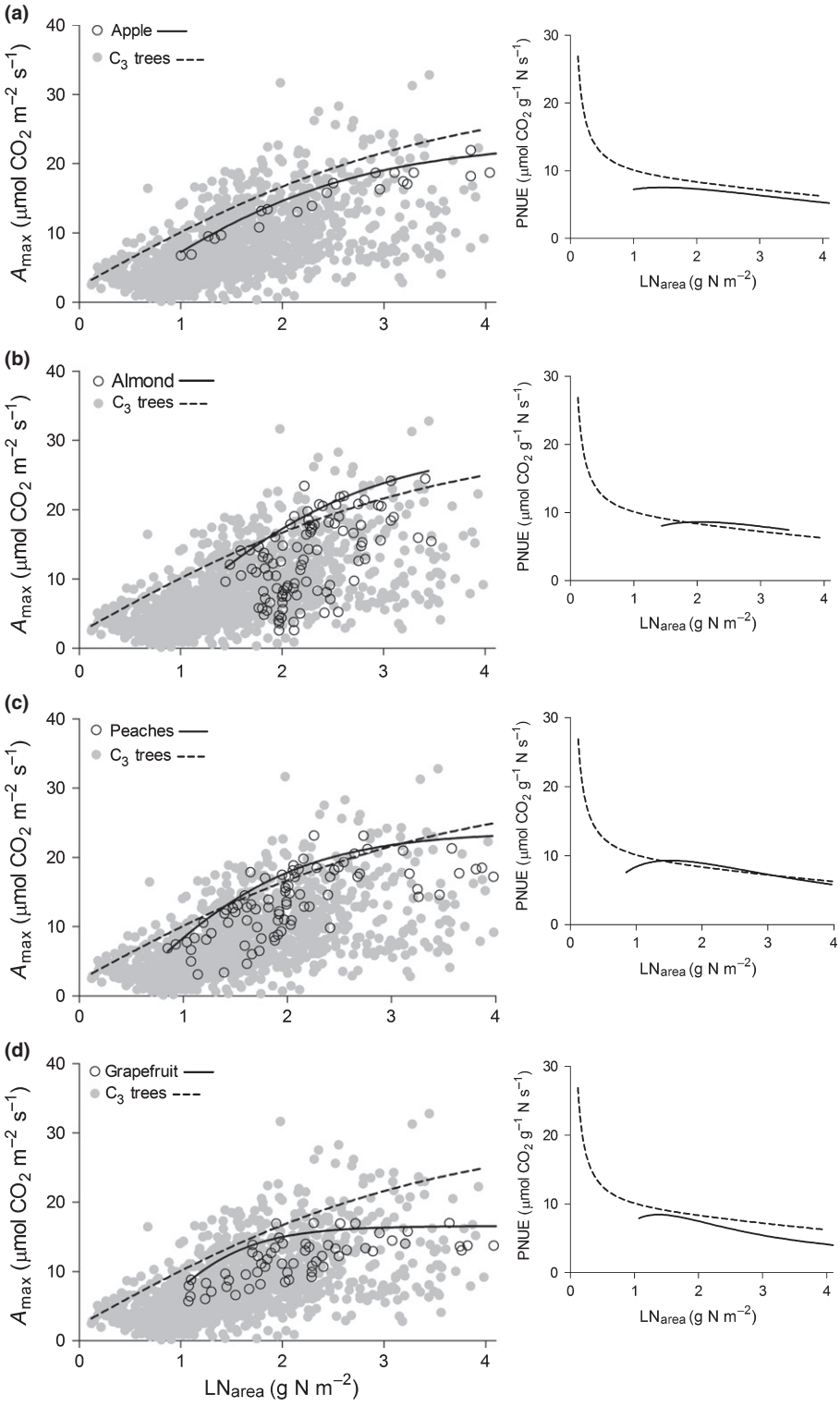


**Fig. 4** Quantile regression models for the relationship between the light-saturated photosynthesis rate ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$) and the leaf nitrogen content ($LN_{area}$; g N $m^{-2}$) for wild $C_3$ trees (dashed line and gray markers) and related cultivated species (solid line and white markers): (a) apple; (b) almond; (c) peaches; (d) grapefruit. Insets: the predicted photosynthetic nitrogen-use efficiency (PNUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$) along the same leaf nitrogen content gradient according to the fitted model.

in a global change scenario remains to be determined. There is evidence that increased $CO_2$ concentration has a positive effect on $A_{max}$ in $C_3$ species (Ainsworth & Long, 2005; Tubiello *et al.*, 2007). The relative responses of $A_{max}$ to increased $CO_2$ were similar in $C_3$ crops and $C_3$ grasses (Ainsworth & Long, 2005; Tubiello *et al.*, 2007). Therefore, we can speculate that increases in PNUE associated with increased $A_{max}$ would be similar for these groups of species, maintaining the differences currently observed. However, this positive effect of $CO_2$ on $A_{max}$ could be cancelled to some degree by increases in temperature (Lobell & Gourdji, 2012).

Maize and sorghum had lower PNUE values, $< 1$ g N $m^{-2}$, compared with $C_4$ wild grasses. Muchow & Sinclair (1994) showed that, in general, maize and sorghum operate at different

$LN_{area}$ (1.8 and 1.3 g N $m^{-2}$ in maize and sorghum, respectively). In both cases, these $LN_{area}$ values are within the range of those determining similar PNUE values to $C_4$ wild grasses.

The $C_3$ dicotyledonous annual crops had similar PNUE values to the $C_3$ wild herbs, at least from values of *c.* 1 g N $m^{-2}$ upwards. The exceptions were soybean and potato, having lower PNUE than the wild $C_3$ herbs in a wide range of $LN_{area}$. Soybean leaves are N storage organs synthesizing vegetative storage protein (Staswick, 1994). This protein can account for almost 50% of total soluble protein in the leaves; the presence of these N-rich proteins may help to explain the low PNUE observed for this crop. Potato, on the other hand, has been reported as a species having inherently low leaf PNUE than other crops (Vos & van der Putten, 1998). The low PNUE can also explain the low NUE observed at crop level (Ospina *et al.*, 2014).

The $C_3$ cultivated trees were not different in their PNUE response to $LN_{area}$ from the $C_3$ wild woody species. Because of the long times required for commercial tree breeding, it was expected that there would not be meaningful differences from their wild counterparts. One interesting aspect of this comparison is that the $LN_{area}$ of crop trees was never < 1 g N $m^{-2}$, as it was for the wild trees. One hypothesis we propose to explain this finding is that farming ensured more N availability to crop trees, whereas wild trees grow and evolve in climax communities characterized by low availability of soil nutrients, especially those from tropical or subtropical rainforests (Thompson *et al.*, 1992; Reich *et al.*, 1994). In addition, it was reported that the leaf N concentration changed among tree species according to the optimal functioning for C fixation related to the shade tolerance syndrome (Niinemets & Tenhunen, 1997); the wild tree database included some understory species that had low $LN_{area}$ associated with this syndrome. Another hypothesis to explain lower $LN_{area}$ in wild vs cultivated trees has to do with seasonal variations in temperature and soil water content (Muller *et al.*, 2011; Sugiura & Tateno, 2011); for example, wild tree measurements in the dry season in tropical regions may have reduced $LN_{area}$. Leaf N also depends on the amount of radiation in a vertical gradient of light incident across a dense canopy (Hikosaka, 2016); wild trees may be adapted to reduced amounts of radiation (and hence invest lower $LN_{area}$) as they evolved in dense canopies. In the case of cultivated trees, these species are grown in near isolation, without understory competition from herbaceous communities, and with supplemental fertilization (Tagliavini *et al.*, 1995; Zarate-Valdez *et al.*, 2015). Also, chemical protection in commercial orchards prevented pests and diseases that are more prone to occur in leaves with greater N concentrations (Coley *et al.*, 1985).

Findings from our work have implications in both the agricultural and ecological domains. In the agricultural domain, securing food demand requires understanding of ecophysiological constraints to increase C fixation of cultivated species (FAO, 2009; Tilman *et al.*, 2011). Our results show almost no advantages for cultivated species to fix more C in the physiological frontier along a wide range of N leaf contents when compared with wild species. There is evidence, however, that photosynthesis is not fully optimized, suggesting that there are possibilities to increase crop yields by improving this process (e.g. Murchie *et al.*, 2009). In the near future, it is likely that novel biotechnologies will explore alternative ways to increase $A_{max}$ and PNUE at the cell level, including synthetic C fixation pathways (Bar-Even *et al.*, 2010; Raines, 2011; McGrath & Long, 2014) able to move up this physiological frontier. In the ecological domain, C fixation and sequestration among wild species in natural terrestrial ecosystems have a prominent role to play in attenuating the consequences of current climate change (Lai, 2004; Davidson & Janssens, 2006).

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## Author contributions

J.L.R. and P.A.C. designed the research, compiled the database, analyzed the data, and wrote the paper.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Table S1** Bibliographic references included in the meta-analysis

**Table S2** Dataset included in the meta-analysis

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